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(54) Title: TOPICAL APPLICATION OF AMILORIDE OR ANALOGUES THEREOF FOR TREATMENT OF INFLAMMATION

(57) Abstract

The treatment of inflammatory skin and eye disorders by the topical application of amiloride or its analogues.

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TOPICAL APPLICATION OF AMILORIDE OR ANALOGUES THEREOF FOR TREATMENT OF INFLAMMATION

Background of the Invention

Field of the Invention

This invention relates to the topical therapy of inflammatory skin and eye disorders.

Known treatments of disorders of dermal inflammation and cellular proliferation in the skin include systemic treatment with glucocorticoids, antimetabolites, retinoids, cyclosporine, and phototherapy, including the use of psoralens combined with ultraviolet A radiation.

For topical therapy of many of the acute and chronic inflammatory and proliferative diseases of the skin, such as allergic contact dermatitis, treatment typically involves the application of glucocorticosteroids. The mechanism(s) of action of these agents are not fully understood.

Corticosteroid therapy has been associated with several adverse side effects, including hypothalmic-pituitary adrenal axis suppression, Cushings Syndrome, growth retardation, epidermal and dermal atrophy, hypopigmentation, and exacerbation of underlying diseases. When applied to the eye topically, glucocorticoids can produce a number of adverse side-effects including ocular hypertension, posterior subcapsular cataract, thinning of the cornea, defects in visual acuity and field of vision, penetration of the

globe, or secondary infection pathogens including Herpes simplex.

It is known from in vitro studies that the potassium sparing diuretic amiloride, which is a pyrazine derivative, and its analogues can inhibit ion transport processes at the cell membrane. Modification of ion transport at the cell membrane has been shown to alter cellular activities. Such modifications are known in some circumstances to inhibit cellular proliferation.

For example, amiloride and a number of its analogues having structural modifications at ring positions 2, 5, and 6 have been investigated in vitro for their activity in inhibiting proton and sodium exchange by Vigne et al. in "Structure-Activity Relationships of Amiloride and Certain of its Analogues in Relation to the Blockade of the Na^+/H^+ Exchange System," Molecular Pharmacology, 25, P. 131, 1984.

Rijzewijk et al. in "Recruitment of Quiescent (G.) Cells Following Epidermal Injury is Initiated by Activation of the Phosphoinositol Cyle", Journal of Investigative Dermatology, vol, 90, p.44, 1988, suggest the proliferative response to injury of the epidermis is a result of activation of the phosphoinositol cycle. The activation may be inhibited by inhibiting Na^+ transport which in turn may be accomplished by topically applying amiloride in vivo after removal of the stratum corneum.

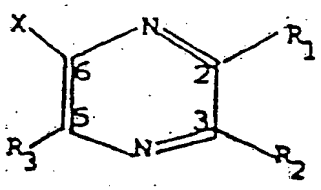
Other sites of amiloride activity which have been observed in vitro include sodium-calcium exchange systems, and specific inhibition of a low threshold voltage gated calcium channel in neural cells. In vitro biological activities of amiloride include inhibition of lectin-induced human T cell proliferation, phorbol ester induced murine thymocyte proliferation, immunoglobulin production by human peripheral mononuclear cells, differentiation of human leukemic cells, arachidonic acid release by human platelets and inhibition of stimulus provoked fibroblast DNA synthesis.

Investigations have also associated the inhibitory action of amiloride on ion transport processes with the drug's biological activity. It has also been suggested that the antiproliferative action of amiloride may also include its ability to inhibit protein synthesis, intercalate within DNA, and inhibit DNA topoisomerase II.

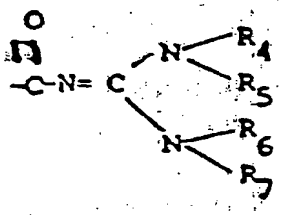
Yu et al. in U.S. 4,067,975 discloses some pyrazinamides which can be used topically to treat psoriasis, a disorder characterized chiefly by hyperproliferation of the epidermis.

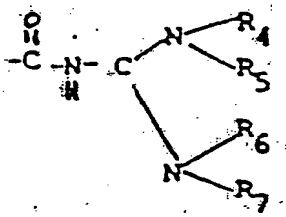
Summary of the Invention

A method is provided for treating inflammation in a mammal comprising topically applying to the mammal an inflammation reducing amount of a compound having the formula:

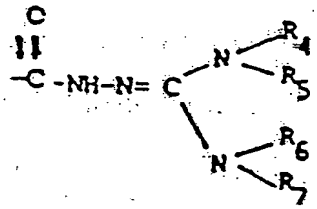


wherein R_1 is carboxyl, $-(NH_2)_2CNNHNH_2$,



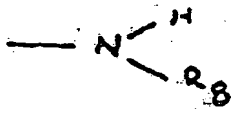


or



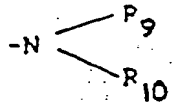
where each of R_4, R_5, R_6, R_7 independently is H, a lower alkyl, phenyl, phenyl lower alkyl, or lower alkyl being substituted with phenyl, halogen substituted phenyl, naphthyl or hydroxy;

R_2 is



where R_8 is H, lower akyl, lower alkoxy or lower alkyl substituted with phenyl or furan,

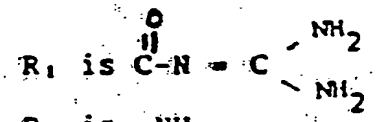
R_3 is H or:



wherein each R_9 and R_{10} independently is H, a lower alkyl, lower alkoxy, lower alkenyl, lower cycloakyl, or lower alkyl being substituted with furan, pyran, hydroxy, or a halogen, primary amino, or - (NH=)C-NH_2, and X is H or a halogen. A lower alkyl, alkoxy and the like, as defined herein are those having 1-6 carbon atoms, inclusive.

In a preferred embodiment, the compound is amiloride.

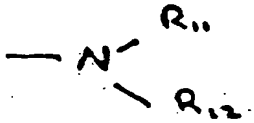
In other preferred embodiments, the compound has the structure where



R_2 is $-NH_2$,

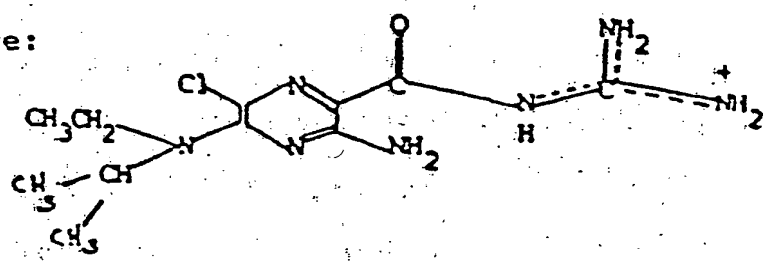
x is Cl, and

R_3 is

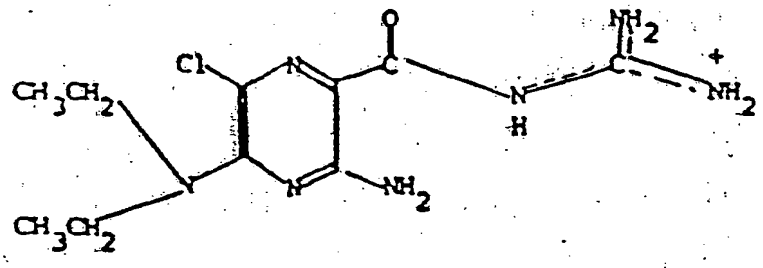


wherein each R_{11}, R_{12}, is selected from the group consisting of lower alkyls or lower alkenyls.

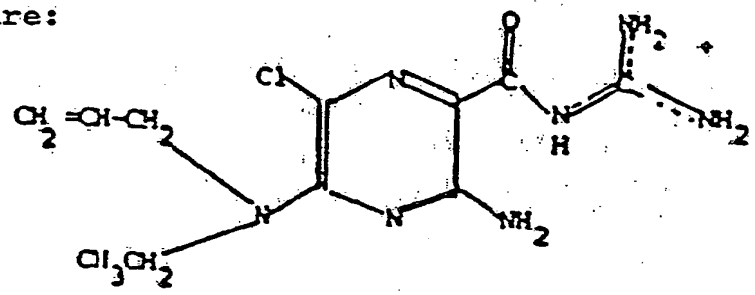
In another preferred embodiment, the compound has the structure:



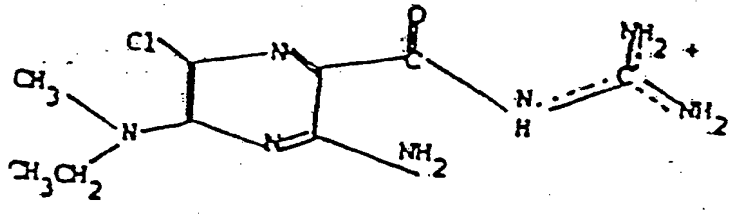
In another preferred embodiment, the compound has the structure:



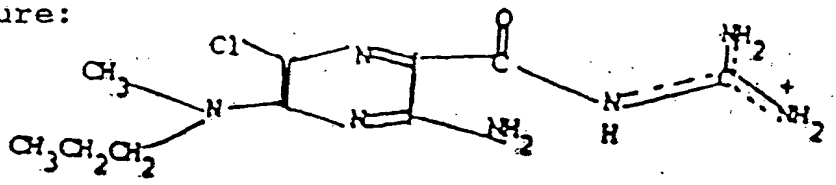
In another preferred embodiment, the compound has the structure:



In another preferred embodiment, the compound has the structure:



In another preferred embodiment, the compound has the structure:



In some preferred embodiments, a compound of the invention is applied to the stratum corneum for treatment of inflammation arising from allergic contact dermatitis, irritant dermatitis (non allergic contact dermatitis), acne vulgaris, acne rosacea, seborrheic dermatitis, atopic dermatitis, cutaneous lupus erythematosus, lichen planus, or exposure to ultraviolet radiation.

In some embodiments, the compound of the invention may be mixed in a stratum corneum transport facilitating vehicle prior to applying.

In other preferred embodiments, the compound of the invention is applied to the eye for the treatment of allergic conjunctivitis, irritant conjunctivitis, viral conjunctivitis, acne rosacea, keratitis, uveitis, scleritis, or episcleritis.

In some embodiments, the compound is prepared in a suitable vehicle for application to the eye.

Another aspect of the invention provides a method for inhibiting cutaneous cellular ion transport processes by topically applying a therepeutically effective amount of an amiloride or an amiloride analogue capable of inhibiting cellular ion transport.

Another aspect provides a method of treating inflammation in a mammal comprising topically applying to the mammal an inflammation reducing amount of amiloride or an amiloride analogue capable of inhibiting cellular ion transport.

Yet another aspect of the invention provides a method for inhibiting cellular proliferation by inhibition of cytokine release by topically applying a therapeutically effective amount of amilorides or an amiloride analogue capable of inhibiting cytokine release.

Another aspect provides a method for treating inflammation in a mammal comprising topically applying to the mammal an inflammation reducing amount of amiloride or an amiloride analogue capable of inhibiting cytokine release.

The invention provides a new topical treatment for a variety of cellular proliferative, and inflammatory diseases of the skin and eye while avoiding side effects associated with prior methods such as corticosteroid application.

Description of the Preferred Embodiment

The drawings are first described.

Brief Description of the Drawings

Fig. 1 is a graph showing the effect of topical application of amiloride hydrochloride on the murine ear swelling response to trinitrochlorobenzene (TNCB) in mice.

Fig. 2 is a light micrograph of the ear of a mouse 48 hours after trinitrochlorobenzene application and treated with base cream absent amiloride.

Fig. 2a is a light micrograph showing an ear as in Figure 2 but treated with 1% amiloride hydrochloride.

Fig. 3 is a graph showing dose response relationship of the trinitrochlorobenzene induced mouse ear swelling response to topical amiloride hydrochloride.

Fig. 4 is a graph showing dose response relationship of the dinitrofluorobenzene induced murine ear swelling response to topical amiloride hydrochloride.

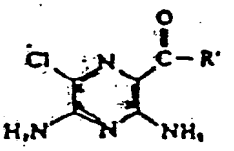
Fig. 5 is a graph showing time course of ear swelling after ultraviolet exposure and application of amiloride at various time points.

Structure

Amiloride consists of a substituted pyrazine ring having an acetylguanidine group at ring position 2, amino groups at ring positions 3 and 5 and a chloride is attached at ring position 6. Amiloride analogues include a number of compounds with a variety of modifications at

ring positions 2, 3, 5 and 6. The term amilorides (an amiloride), as used herein, refers to amiloride and its analogues.

Among the amiloride analogues which can be useful for treatment of inflammation are those capable of inhibiting sodium transport across cell membranes. Tables 1-4, taken from Vigne, supra, illustrate amiloride analogues having inhibiting effects on the Na^+ transport system.



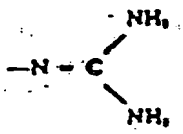
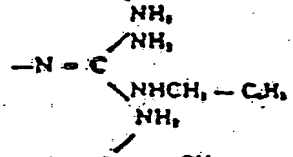
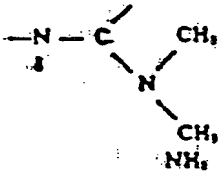
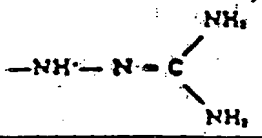
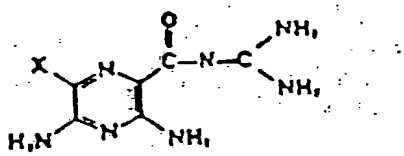
	R'
Amiloride	
Compound 1	
Compound 2	
Compound 3	

Table 1 (from Vigne): Amiloride analogues having substitutions on the guanidino group.

In general, it has been found, as discussed in Vigne, that the unsubstituted guanidino group is important for high activity of the molecule. Its modification results in less active molecules. The guanidino group of amiloride is thought to recognize a Na^+ site on the Na^+/H^+ exchanger.



	X
Compound 4	F
Amiloride	Cl
Compound 5	Br
Compound 6	I

Table 2 (from Vigne): Amiloride analogues having halogen substitutions at position 6.

The nature of the 6-halo group is not important for the Na^+ inhibition activity of amiloride. Only when fluoro was substituted for chloro does the compound lose some activity.

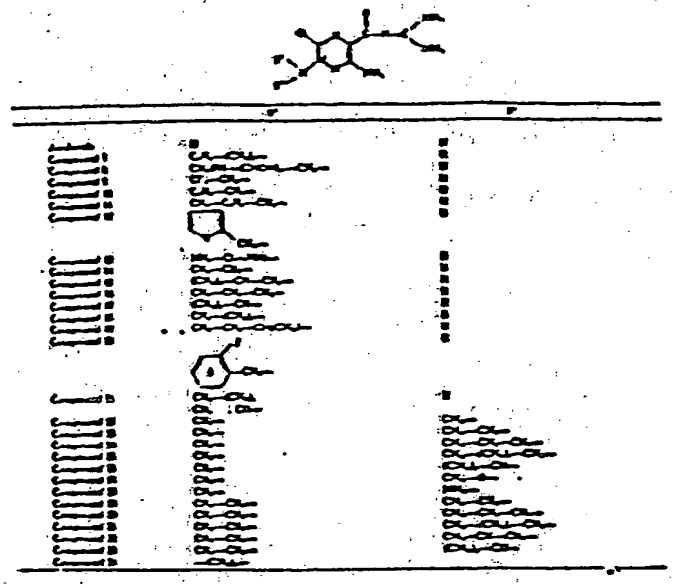
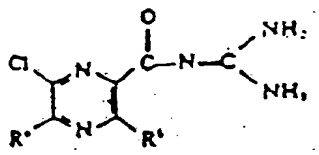


Figure 3 (from Vigne): Amiloride analogues having various substitutions in the 5-amino group.

The potency of amiloride derivatives for Na^+ inhibition can be increased by substitution of the 5-amino group. Monosubstituted derivatives generally had about the same activity as amiloride irrespective of the size of the substituent. On the other hand, disubstitited derivatives for example, those including substitution with lower alkyls, that is, having from 1-6 carbon atoms, exhibited as much as 140 times the potency of amiloride.



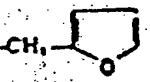
	R'	R'
Amiloride	NH_2	$-NH_2$
Compound 35	H	$-NH_2$
Compound 36	H	$-HN-CH_2$ 
Compound 37	H	$-HN-CH_2-C_6H_5$
Compound 38	CH_2-CH_2-NH-	$-HN-CH_2-CH_2-O-CH_3$

Table 4 (from Vigne): Amiloride analogues having substitutions at positions 3 and 5.

The role of the 3-amino group substituents in Na^+ transport inhibition is less clear, mainly because fewer analogues have been tested. However, it seems that substitution of the 3-amino group leaves activity unchanged or produces a decrease.

Other amiloride analogues which inhibit Na^+ transport are disclosed, for example, by Benos et al. in "Effects of Amiloride and Some of Its Analogues on Cation Transport in Isolated Frogskin and Thin Lipid Membranes." J. of General Physiology, 68, 1976, 43, and Cuthbert et al. in "Effects of Some Pyrazine Carbotyamides on Sodium Transport in Frogskin" J. Pharmacol, 3, 1978, 139, which are hereby incorporated by reference.

Use

Topical application of the amilorides can be used for treatment of inflammatory skin disorders involving a cutaneous inflammatory response such as those in the dermis, the lower layer of the skin. Afflictions include allergic contact dermatitis, irritant dermatitis, acne vulgaris, acne rosacea, seborrheic dermatitis, atopic dermatitis, cutaneous lupus erythematosus, lichen planus, mycosis fungoides, as well as other cutaneous infiltrations of benign and malignant inflammatory cells. The application of amiloride or its analogues may also favorably modify the inflammation associated with sunburn by application to sun exposed sites after exposure.

Topical application of amiloride can also be used for treatment of eye disorders. Therapy for ocular inflammation does not require transport through stratum corneum, the main barrier to penetration in the skin, since the conjunctiva and cornea are not cornified. Disorders which may be treated include allergic, irritant, or viral conjuntivitus, acne rosacea, keratitis, uveitis, scleritis, episcleritis, and other inflammatory conditions.

Topical Skin Treatment

Inflammatory cutaneous lesions can be treated topically with amiloride or its analogues at a range of concentrations sufficiently high to produce an antinflammatory effect but not so high as to raise

systemic amounts to toxic levels. For example, amilorides may be applied at concentrations between 0.5 and 2.0% w/w in an appropriate vehicle (such as hydrated petrolatum). Preferably, the vehicle facilitates penetration of amiloride by increasing the permeability the stratum corneum. These include, for example, dimethylsulfoxide, decylmethyl sulfide, propylene glycol-isopropyl alcohol, laurocaram, Vehicle N (Neutragena Corp. Los Angeles, CA), hydrated petrolatum, and ethanol. Application can be performed four times per day, with or without occlusion, until a satisfactory clinical response has occurred.

The therapeutic efficacy of amiloride as a topical anti-inflammatory agent was examined in the murine ear swelling response as a model of cutaneous inflammation. The tests included treatment of induced allergic contact dermatitis (ACD) and uv radiation (UVR) exposure inflammation.

Female mice, 8 to 12 weeks of age, of the BALB/c, A/J, or C57BL/6 strain were purchased (Jackson Laboratories, Bar Harbor, Me) and used in all experiments.

The degree of reaction was quantitated by measurements of ear swelling with a micrometer (Fowler, Biggsfield, England). Measurements were taken immediately before and 24 and 48 hours after application

of the sensitizing agent. All measurement were made by an investigator in a coded fashion.

For sensitization and elicitation of the ACD reaction, the abdomens of BALB/c or A/J mice were shaved with electric clippers, and the mice were sensitized by epicutaneous application of 20 μL of 2% wt/vol 2,4,6-trinitrochlorobenzene (TNCB) at that site in a 4:1 mixture of acetone and corn oil, or 20 μL of 0.5% vol/vol 1-fluoro-2,4-dinitrobenzene (DNFB) in acetone. Sensitization was performed 5 to 7 days before elicitation of the ACD reaction. An ACD reaction was elicited by application of 5 μL of 1% TNCB or 0.2% DNFB to dorsal and ventral surfaces of ears of mice.

Amiloride hydrochloride (sigma Chemical Co., St. Louis, Mo) was dissolved in a small volume of distilled water before thorough mixing with an appropriate weight of hydrated petrolatum (Denison Laboratories, Pawtucket, RI). At various times after elicitation with sensitizing agents or UVR treatment, the minimal volume of amiloride preparation necessary to cover the treated surface completely with a thin film was applied to both surfaces of the ear. A similar application of hydrated petrolatum alone was made to matched groups of mice to serve as a control.

The effect of topical application of 1% wt/wt amiloride on TCNB induced murine ear swelling in sensitized mice is shown in Fig. 1. The sensitization

dose of TNCB was not applied to the negative control group. Positive controls received base cream alone. Amiloride pretreated and amiloride groups received 1% topical amiloride 4 hours before and 1 hour after elicitation, respectively. Data are mean ± standard deviation (SD) of five A/J mice per group, representative of three experiments.

The degree of ear swelling was dramatically decreased relative to the positive control in mice that were treated with topical amiloride 1 and 12 hours after application of TNCB for elicitation of the ACD reaction, and it did not significantly differ from the ear swelling response of nonsensitized mice (negative control). The relative effectiveness of the application of amiloride 1 and 12 hours after elicitation was constant for 24 hours and 48 hours after initial treatment. All five mice in this experiment responded to the application of amiloride. Maximal swelling on all cases occurred within 24 to 48 hours after application of the elicitation dose of TNCB.

Pretreatment of the elicitation site 4 hours before TNCB exposure did not appear to inhibit ear swelling (Fig. 1) relative to the positive control. This suggests that no permanent alteration occurs in the responsiveness of cells at this site, and minimal residual drug is active on the ear 4 hours after amiloride application. Mice were observed to groom

themselves fastidiously after the application of cream to their ears. This may explain the removal of amiloride activity, but it also raises the question of systematic effects of ingested cream. In the treatment protocol used for these experiments, no apparent systematic effects were observed. Furthermore, the application of amiloride cream to one ear did not decrease the ear swelling response of the opposite ear. This suggests that the systemic levels that may be achieved through either absorption or incidental ingestion did not induce the decrease in ear swelling observed after local application.

In Figs. 2-2a, micrographs of ear specimens taken from TNCB treated mice are shown. In Fig. 2, the ear exhibits a large inflammatory cell infiltrate 48 hours after TNCB application. The topical application dramatically reduced the number of mononuclear and polymorphonuclear lymphocytes seen at 48 hours (Fig. 2a). This decrease in the number of inflammatory cells corrolates with the decrease in ear swelling after amiloride application demonstrated in Fig. 1.

The dose response relationship for the TNCB induced ear swelling response to a single application of amiloride is shown in Fig. 3. Positive controls were exposed to hydrated petrolatum alone; negative control mice were not sensitized before application of the elicitation dose. Amiloride was applied 1 hour after

elicitation. Results are 24 and 48 hours after elicitation and represent the mean ± SD of five BALB/c mice per group, representative of three experiments.

In the experiment of Fig. 3, a single application of amiloride was made 1 hour after TNCB application. Maximum inhibition of the ear swelling reaction by a single topical application of amiloride occurred with 2% wt/wt amiloride. Comparison of this single application regimen at a dose of 1% with two applications each at 0 and 12 hours (Fig. 1) demonstrated that the relative effectiveness of a single application is less than that of two applications but remains significant (P =005) relative to treatment with the base cream alone.

The effectiveness of topical amiloride in the inhibition of the murine ACD response was also examined with ear swelling reactions to DNFB (Fig. 4). Amiloride cream was applied 1 hour after elicitation. Results are 24 hours after elicitation and represent the mean ± SD from each ear of five A/J mice per group, representative of two experiments. The treatment protocol after elicitation of the ACD reaction with DNFB was identical in these experiments to those described for TNCB in Fig. 3.

Comparison of Figs. 3 and 4 shows that less ear swelling was induced by DNFB than TNCB. This lesser degree of swelling occurred with both A/J and BALB/c mice. As with TNCB reactions, topical amiloride was

successful in inhibiting the murine ACD reaction to DNFB. However, amiloride was found to inhibit ear swelling by DNFB significantly at lower concentrations than TCNB.

For testing treatment of UVR induced swelling, fluorescent tubes (FS 40; Westinghouse Inc., Bloomfield, NJ) were used as the UVR source for irradiation of mice in these experiments. Irradiance and spectral output were measured with a meter and ultraviolet B (280 to 320 nm) detector (IL 1700; International Light Research, Newburyport, Mass) and spectroradiometer (model 742; Optronic Laboratories, Orlando, Fla.), respectively. Spectral output for the UVR source peaked at 312 nm, with 57% of the output in the ultraviolet B range (200 to 320 nm); 38.6% in the ultraviolet A range (320 to 400 nm), and 4,4% in the ultraviolet C range (200 to 290 nm). Mice were exposed for 3 hours with intermittent rotation every 30 minutes, 25 cm from the overhead light source, at an average irradiance of 3.0×10^{-4} W/cm2, while held individually in specially constructed divided cages with open caged tops. Swelling was evaluated by measurements of ear thickness immediately before and 24 and 48 hours after irradiation.

Ultraviolet radiation will induce a distinct inflammatory response in murine skin, which may be quantified by measurements of ear swelling similar to those described for evaluation of the ACD reaction. The effect of topical amiloride on UVR-induced inflammation

was, therefore, evaluated by serial measurements of ear thickness. Amiloride in aqueous solution strongly absorbs light in the ultraviolet region. Therefore, to avoid effects secondary to UVR absorption, amiloride was applied to the site of interest only after irradiation.

The relative effect of topical amiloride on UVR-induced ear swelling over time is shown in Fig. 5. In Fig. 5, open squares □ indicate base cream alone applied at 0, 12, and 24 hours; closed squares □, 1% amiloride hydrochloride applied at 0 and 12 hours (P.<005) at 24 and 48 hours); and closed circles , 1% amiloride applied only at 24 hours (p.<.05 at 48 hours). Data represent the mean ear swelling of ten C57BL6 mice per group in two separate experiments.

Maximal ear swelling was observed 48 hours after UVR exposure to approximately 3000 mJ/cm2 of ultraviolet B (290 to 320 nm) radiation. Amiloride cream, 2%, applied immediately and 12 hours after irradiation significantly (P.<005) decreased the total ear swelling response measured at 24 and 48 hours (Fig. 5). A single application of amiloride at 12 or 24 hours decreased the relative final effectiveness of amiloride on the final ear swelling response. However, delayed application of amiloride was effective in delaying the rate of increase in ear thickness induced by UVR and significantly (P.<05) descreased the 48 hour ear swelling response.

Topical application of amiloride was shown in the experiments above to inhibit the inflammatory response to contact sensitizing agents in sensitized animals, as well as UVR induced tissue swelling. The response to amiloride seen in these experiments was both rapid and persistent. These observations suggest that local effects on the immune system of the skin can be meditated by topical amiloride application.

Diffusion of amiloride through normal human epidermis and hairless mouse skin was determined by placing clinically normal human abdominal epidermis obtained at autopsy, or normal hairless mouse dorsal skin with fat removed, in a glass diffusion chamber. A 2mM aqueous solution of amiloride hydrochlorine in normal saline was used as the donor solution. Concentrations of amiloride in the receptor chamber were determined by fluorescence spectroscopy. Flux calculations for amiloride through human epidermis showed an average value of 1.083 $nm/cm^2/hr$. Similar calculations for mouse skin yielded a value of 11.86 $nm/cm^2/hr$. These values represent amiloride flux in a non-optimized vehicle for penetration through the stratum corneum.

Topical Eye Treatment

Inflammatory ocular conditions will be treated by topical application of amiloride and its analogues at concentration ranges sufficient to reduce inflammation

but not so high such that systematic levels are toxic. For example, a 0.5 to 2.0% w/w preparation of amiloride or its analogues in appropriate vehicles can be applied to the conjunctival sac of the inflammed eye four times per day intil a satisfactory clinical response occurs. Appropriate vehicles would be sterile, nonirritating, and preferably include an aqueous component for solubilizing amiloride. For example, opthalmic solutions such as Methopto 1/4% Sterile Opthalmic Solution (Professional Pharmacol Co., Amityville, NY) or Duolube (white petrolatum, liquid petrolatum, available from Moro Pharm., Tewksbury, MA) or Lacri-Lube (white petrolatum, 55% mineral oil, 42.5%, nonionic lanolin, 2%, chlorobutanol, 0.5% and available from Allergan, Irvine, CA) or other sterile opthalmic ointments mixed with an aqueous phase of amiloride solution.

Mechanism of Action

Inflammation is a physiological response which may occur as a result of a variety of events within the skin. Initiation and regulation of this inflammatory response is influenced by a number of cellular processes. These events include direct interactions with the cell membrane and the release of soluble molecules which mediate further inflammatory reactions.

We believe that amiloride's ability to inhibit ion transport, is an important step by which cell activation occurs. In particular, we believe that inhibition of

sodium transport, calcium transport, and shifts in cytoplasmic pH, are important in its anti-inflammatory activity. These inhibitory actions are also capable of preventing cell proliferation through specific action on these ion transport phenomena.

It is also possible that Na^+/H^+ exchange is not obligatory for cell proliferation and the antiproliferative actions of amiloride may be attributed to other events associated with amiloride exposure, for example, its ability to inhibit protein synthesis and intercalate within DNA and inhibit DNA topoisomerase II.

Further, we have discovered that amiloride, in vitro, inhibits the release of the pluripotent stimulator of cellular proliferation, Interleukin-3. Thus, proliferation may be inhibited by interrupting cytokine mediated cellular activation systems initiated by keratinocytes or other cells within the skin.

We have also discovered that amilorides inhibit lymphokine release from transformed keratinocytes in vitro and inhibit arachidonic acid release from an immortal murine fibroblast line following exposure to ultraviolet radiation.

Amiloride, when applied topically to the stratum corneum is absorbed through the epidermis to the region of the dermis where it may act directly by any of the above discussed mechanisms. It is also possible that action in the epidermis, for example, inhibition of

cytokine release may contribute to its effective treatment of cellular proliferation in the skin.

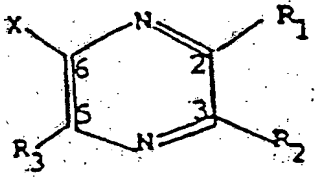
Any or all of these processes may be contributing to the effectiveness of topical amiloride in inhibiting cutaneous inflammation.

It will be understood by those skilled in the art that many variations over the teachings above may be implemented without departing from the spirit and source of the present invention. For example, amiloride analogues which inhibit proliferation by mechanisms other than by inhibiting Na^+ ion transport can be used topically to treat inflammation according to the invention.

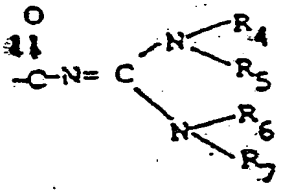
Claims

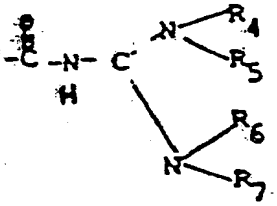
1. A method for treating inflammation in a mammal comprising:

topically applying to said mammal an inflammation reducing amount of a compound having the formula:

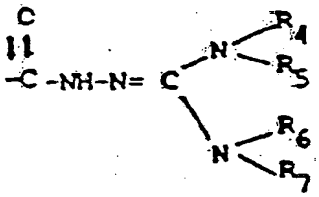


wherein R_1 is carboxyl, $(NH_2)_2CNHNH_2$,





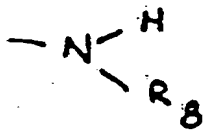
or



where each of R_4, R_5, R_6, R_7 independently is H, a lower alkyl, phenyl, phenyl-lower alkyl, lower alkyl

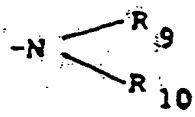
substituted with phenyl, halogen substituted phenyl, naphthyl or hydroxy;

R_2 is:



where R_8 is H, lower akyl, lower alkoxy or lower alkyl substituted with phenyl or furan,

R_3 is H or:

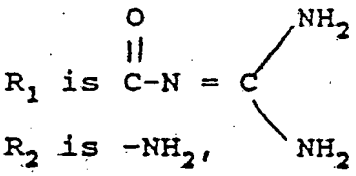


wherein each R_9 and R_{10} independently is H, a lower alkyl, lower alkoxy, lower alkenyl, or lower cycloakyl, or lower alkyl being substituted with furan, pyran, hydroxy, a halogen, primary amino, or $(NH=)C-NH_2$, and

X is H or a halogen.

2. The method of claim 1 wherein said compound is amiloride.

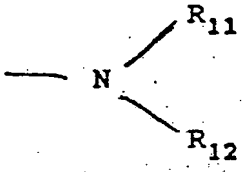
3. The method of claim 1 wherein



R_2 is $-NH_2$,

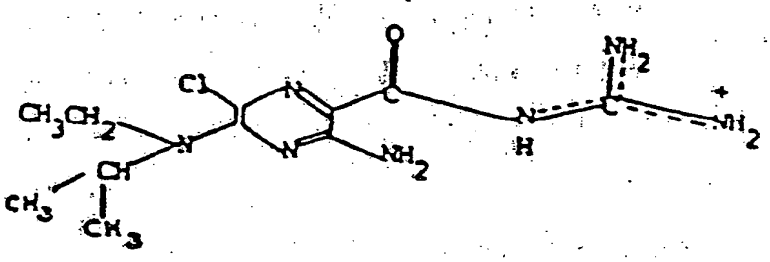
X is Cl, and

R_3 is

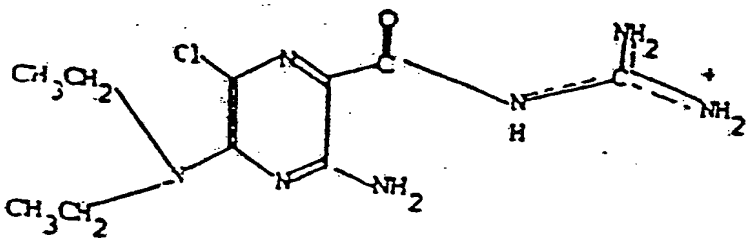


wherein each R_{11}, R_{12}, is selected from the group consisting of lower alkyls or lower alkenyls.

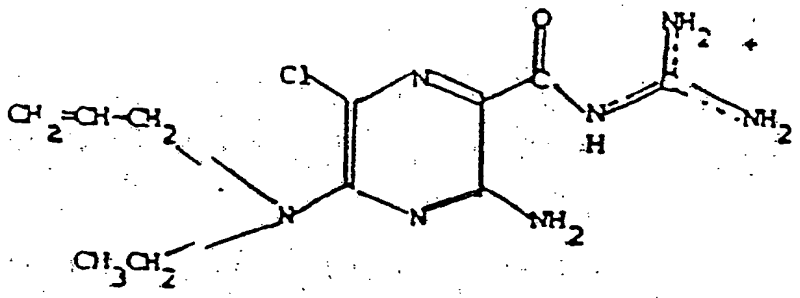
4. The method of claim 3 wherein said compound has the structure:



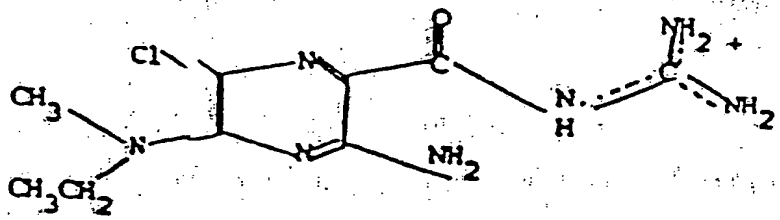
5. The method of claim 3 wherein said compound has the structure:



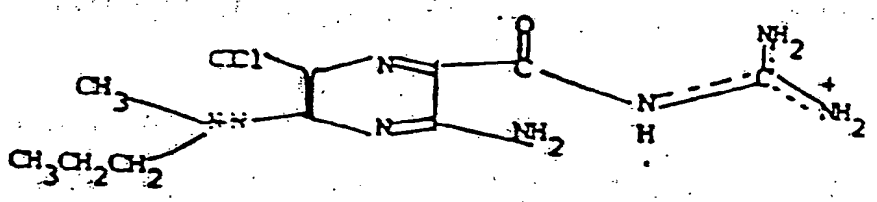
6. The method of claim 3 wherein said compound has the structure:



7. The method of claim 3 wherein said compound has the structure:



8. The method of claim 3 wherein said compound has the structure:



9. The method of claim 1 wherein said applying comprises applying to the stratum corneum.

10. The method of claim 9 wherein said method further comprises facilitating prior to said applying,

mixing said compound with a stratum corneum transport vehicle.

11. The method of claim 9 wherein said inflammation arises from allergic contact dermatitis.

12. The method of claim 9 wherein said inflammation arises from irritant dermatitis.

13. The method of claim 9 wherein said inflammation arises from acne vulgaris.

14. The method of claim 9 wherein said inflammation arises from acne rosacea.

15. The method of claim 9 wherein said inflammation arises from seborrheic dermatitis.

16. The method of claim 9 wherein said inflammation arises from atopic dermatitis.

17. The method of claim 9 wherein said inflammation arises from cutaneous lupus erythematosus.

18. The method of claim 9 wherein said inflammation arises from lichen planus.

19. The method of claim 9 wherein said inflammation arises from exposure to ultraviolet radiation.

20. The method of claim 1 wherein said applying comprises applying said compound to the eye.

21. The method of claim 20 further comprising preparing said compound in an appropriate vehicle.

22. The method of claim 20 wherein said inflammation arises from allergic conjunctivitis.

23. The method of claim 20 wherein said inflammation arises from irritant conjunctivitis.

24. The method of claim 20 wherein said inflammation arises from viral conjunctivitis.

25. The method of claim 20 wherein said inflammation arises from acne rosacea.

26. The method of claim 20 wherein said inflammation arises from keratitis.

27. The method of claim 20 wherein said inflammation arises from uveitis.

28. The method of claim 20 wherein said inflammation arises from scleritis.

29. The method of claim 20 wherein said inflammation arises from episcleritis.

30. A method for inhibiting subcutaneous cellular ion transport processes comprising

topically applying a therepeutically effective amount of amiloride or an amiloride analogue capable of inhibiting cellular ion transport.

31. A method of treating inflammation in a mammal comprising topically applying to said mammal an inflammation reducing amount of amiloride or an amiloride analogue capable of inhibiting cellular ion transport.

32. A method for inhibiting cellular proliferation by inhibition of cytokine release comprising

topically applying a therapeutically effective amount of amiloride or an amailoride analogue capable of inhibiting said cytokine release.

33. A method for treating inflammation in a mammal comprising topically applying to said mammal an

inflammation reducing amount of amiloride or an amiloride analogue capable of inhibiting said cytokine release.

Fig 1

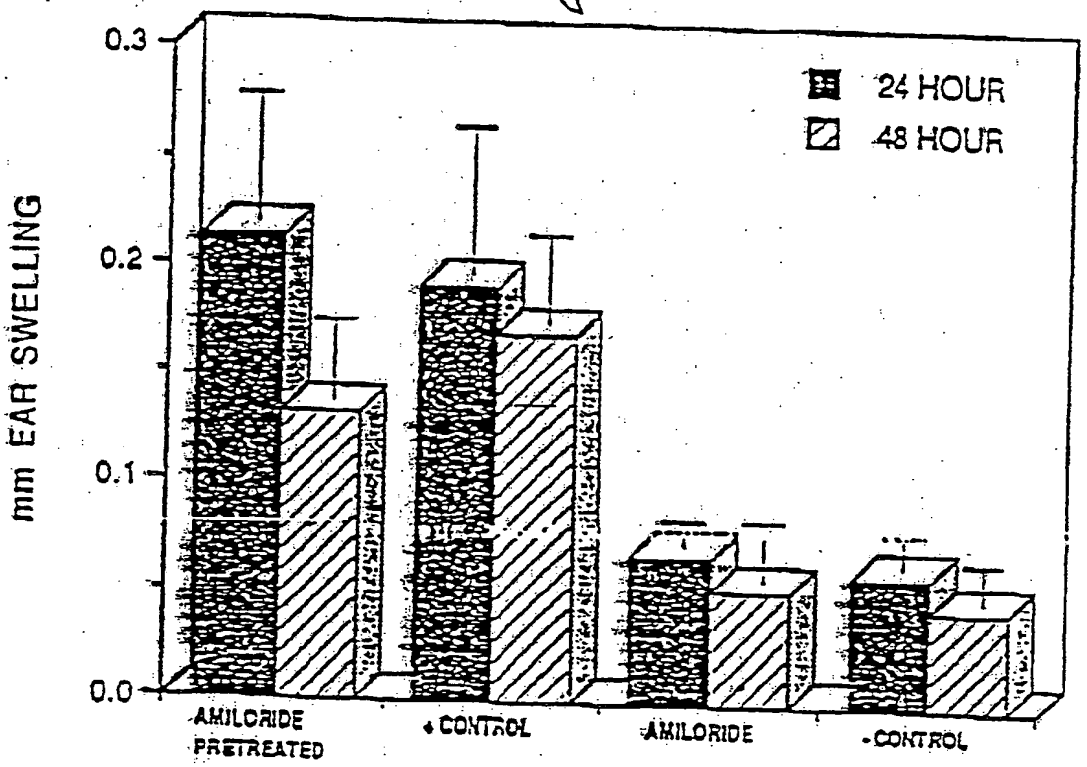

0.3
0.2
0.1
0.0
mm EAR SWELLING
24 HOUR
48 HOUR
AMILORIDE PRETREATED
+CONTROL
AMILORIDE
-CONTROL

Fig 2a

Fig 2

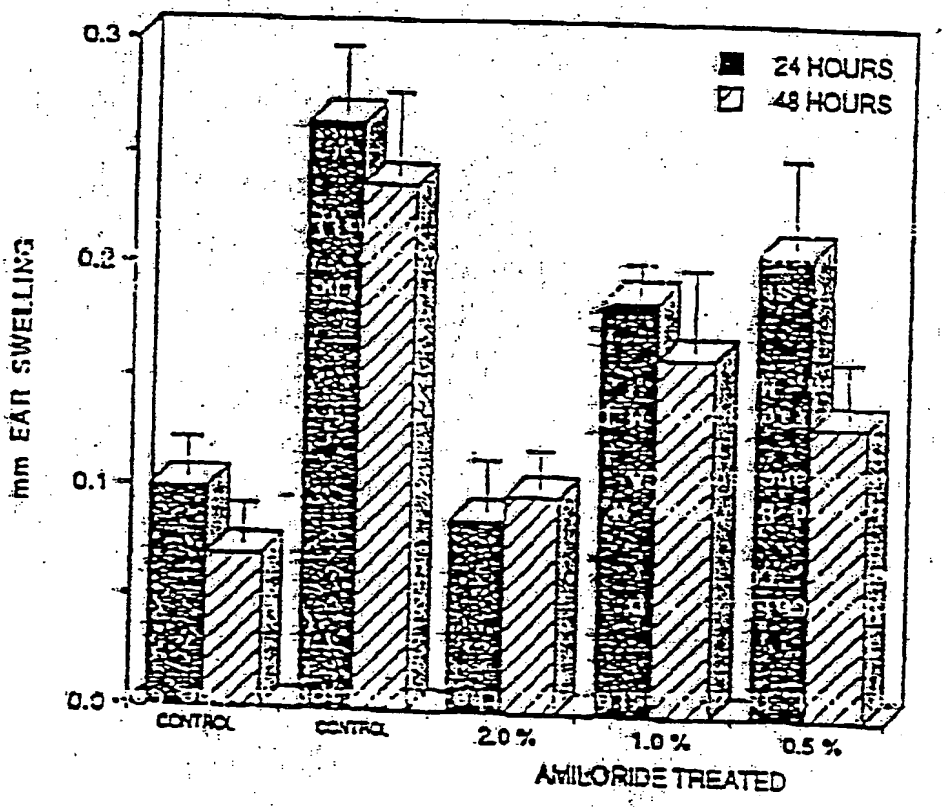

0.3
0.2
0.1
0.0
mm EAR SWELLING
24 HOURS
48 HOURS
CONTROL
CONTROL
2.0 %
1.0 %
0.5 %
AMILORIDE TREATED

Fig 3

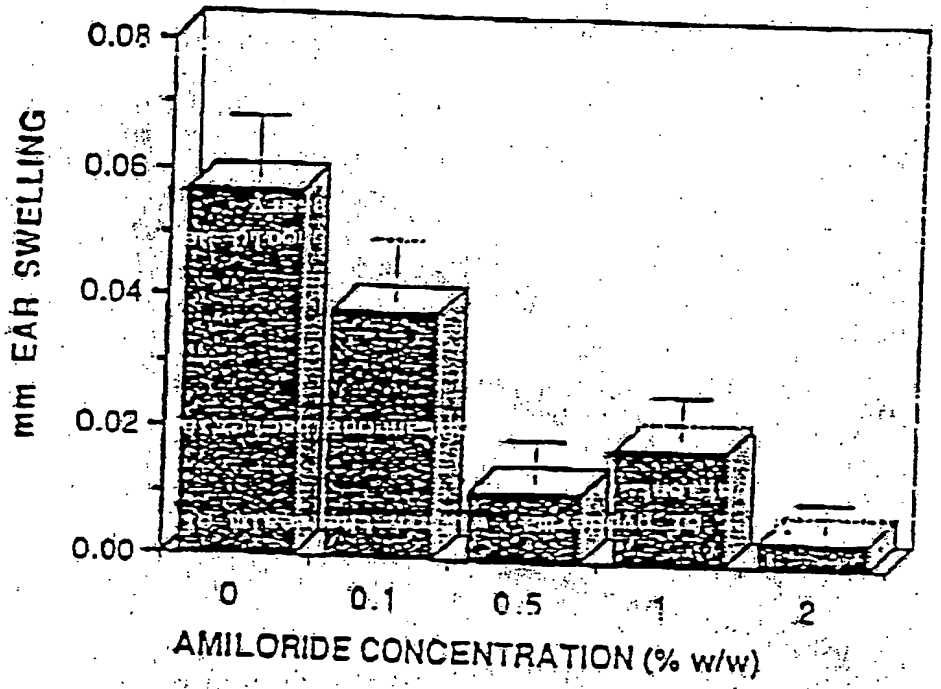

0.08
0.06
0.04
0.02
0.00
mm EAR SWELLING
0
0.1
0.5
1
2
AMILORIDE CONCENTRATION (% w/w)

Fig 4

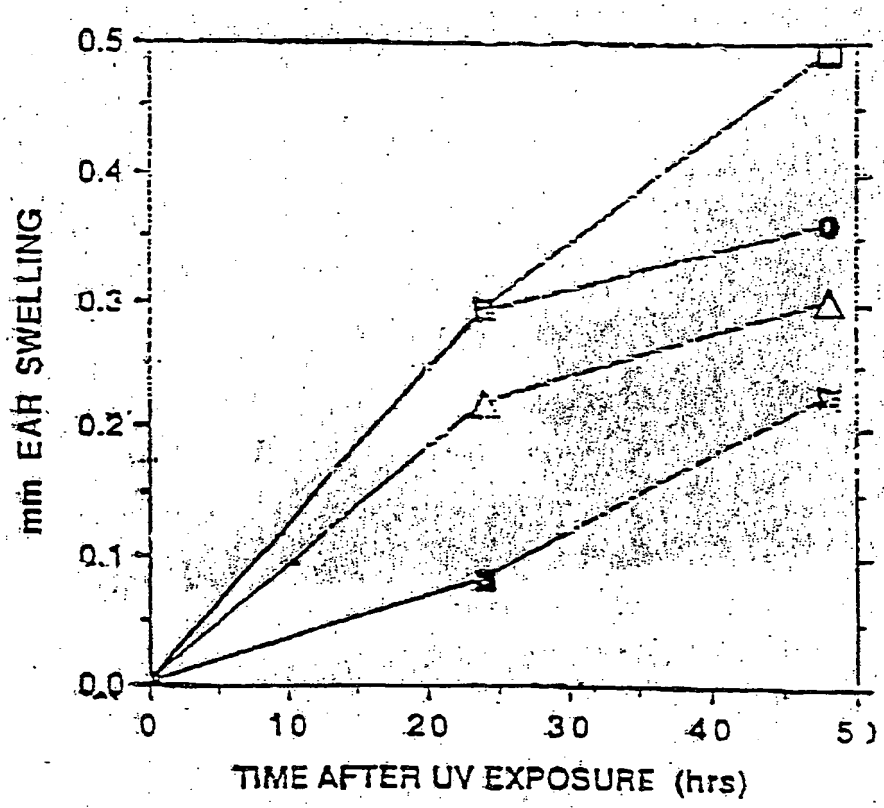


Fig 5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/01224

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5) : A61K 31/495
U.S. Cl : 514/255

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/255

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

CAS ON LINE

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Chemical Abstracts, Volume 90, Number 25, issued 18 June 1979, Columbus, Ohio, USA, Merde and Company, Inc. Amiloride and its 6-substituted derivatives, Japan Kokai Tokkyo 79 12,389.	1-33

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
01 May 1990	20 JUN 1990
International Searching Authority	**Signature of Authorized Officer**
ISA/US	for Stanley J. Friedman Ho Nguyen — HO NGUYEN

Form PCT/ISA/210 (second sheet) (Rev.11-87)